Cell division cycle associated 1 as a novel prognostic biomarker and therapeutic target for oral cancer.

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別紙様式３（課程博士・論文博士共用）

論 文 内 容 要 旨

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学位論文題目	Cell division cycle associated 1 as a novel prognostic biomarker and therapeutic target for oral cancer 口腔癌の予後予測バイオマーカーおよび治療標的分子としての CDCA1 の解析		

Objective

This study aims to identify and characterize novel prognostic biomarkers and therapeutic targets for oral cavity carcinoma (OCC).

Methods

To identify novel prognostic biomarkers and therapeutic targets for human cancers, we performed genome-wide gene expression analysis of various solid tumor tissues and selected candidate genes as molecular targets for OCC. We validated by immmunohistochemical analysis the relationship of these genes product expression with prognosis for 99 OCC patients. In addition, we examined the roles of the candidate proteins for the growth, invasion, and survival of OCC by siRNAs experiments. During this process, we identified cell division cycle associated 1 (*CDCA1*) as a possible prognostic biomarker and therapeutic target for OCC.

Results

Immunohistochemical analysis confirmed that CDCA1 protein was expressed in 67 of 99 OCC tissues (67.7%), but not in healthy oral epithelia. Age factor (higher in ≥65 years; $P = 0.0063$ by Fisher's exact test), pT factor (higher in T3-T4; $P = 0.0112$ by Fisher's exact test), and pN factor (higher in N1 and N2; $P = 0.0282$ by Fisher's exact test) were significantly related to strong CDCA1 expression. Furthermore, CDCA1 expression was significantly associated with poor prognosis for OCC patients ($P = 0.0244$ by log-rank test). In addition, flow cytometric analysis and various apoptosis assays demonstrated that CDCA1 knockdown by siRNAs significantly induced apoptosis probably through the activation of mitotic pro-phase and apoptosis-related protein such as Bad, Bax, cleaved caspase-3, and TRAIL R2/DR5.

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等を用いて印字すること。

２．※印の欄には記入しないこと。

別紙様式３の２（課程博士・論文博士共用）

（続紙）

Discussion

Molecular targeted therapies for OCC are currently being developed. These may provide better efficacy with fewer side effects by specifically targeting cancer-related mechanisms. Current anticancer agents can prolong the overall survival of OCC patients, but they have limited efficacy and induce drug resistance. Therefore, new therapeutic target drugs for OCC are essential for improving the clinical outcome for patients.

In this study, CDCA1 protein was overexpressed in OCC tissues and its overexpression was significantly associated with poor prognosis. To examine mechanism of CDCA1 activation in OCC, we collected comparative genome hybridization and genome sequencing data for CDCA1 using public databases. Missense mutations in CDCA1 were detected in 0.78% of OCC tissues (2/255 cases), but no CDCA1 gene amplification or translocation was reported. Therefore, CDCA1 overexpression could be involved in an epigenetic mechanism contributing to OCC.

In our study, suppression of CDCA1 expression significantly inhibited OCC cell growth and induced apoptosis in OCC cells, but the specific mechanisms remain unclear. According to our findings and previous studies, possible mechanisms exist for CDCA1-mediated regulation of OCC growth and survival: (1) stable spindle microtubule-kinetochore attachment in mitotic prophase of highly proliferative cancer cells; (2) regulation of apoptosis pathways. CDCA1 plays a pivotal role in stable spindle microtubule-kinetochore attachment. Decreased CDCA1 expression inhibited kinetochore attachment to spindle microtubules, resulting in aberrant chromosome segregation, prolonged mitotic blockade, and cell death. In agreement with previous findings, CDCA1 knockdown in OCC cells resulted in impaired growth and apoptosis in cells. Downregulation of CDCA1 increased the expression of apoptosis-related proteins in OCC cells, including Bad, Bax, cleaved caspase-3, and TRAIL R2/DR5. The data suggested the dysregulation of appropriate cell division and subsequent induction of apoptosis of OCC cells through various mechanisms such as mitochondrial-dependent and caspase-dependent pathways.

Conclusion

CDCA1 is likely to play a significant role in OCC carcinogenesis. Importantly, CDCA1 is associated with growth and survival of OCC cells. Since CDCA1 was not expressed in healthy tissues, apart from the testis, it could be a highly specific cancer biomarker. In addition, targeting CDCA1 will lead to the development of new type of therapeutic agents for OCC, such as immunotherapies as well as molecular targeted drugs.

別紙様式８（課程博士・論文博士共用）

学位論文審査の結果の要旨

整理番号	769	氏　　名	Phung Manh Thang
論文審査委員			

（学位論文審査の結果の要旨）※明朝体１１ポイント、６００字以内で作成のこと

本論文では、治療後再発率が高い口腔内扁平上皮癌に対し cell division cycle associated 1（以下、CDCA1 と略）の発現と臨床病理学的因子を加えた予後との関連を検討するとともに、新規治療法の標的分子となりうるかを検討し、以下の点を明らかにした。

１） 口腔内扁平上皮癌細胞株では CDCA1 の mRNA 発現が見られるが、正常口腔上皮では発現がないこと。
２） 口腔内扁平上皮癌組織でも細胞株と同様の結果が見られること。
３） 組織免疫染色で CDCA1 強発現の症例は弱～無の症例に比して有意に予後不良であること。
４） CDCA1 発現は pT 因子、ｐN 因子に相関していること。
５） CDCA1 をノックダウンすると細胞増殖能が低下すること。
６） CDCA1 をノックダウンすると、細胞周期が subG1 期の細胞や、Annexin V 陽性細胞が増加し、Caspase-3/7 の発現が見られること。
７） CDCA1 をノックダウンすると、BAD や BAX 等のアポトーシス誘導タンパクが増加し、アポトーシスをきたす像が得られること。

本論文は、口腔内扁平上皮癌における CDCA1 の意義について予後予測因子としてだけでなく、CDCA1 を標的分子とした新規治療法の可能性につき新たな知見をあたえたものであり、また最終試験として論文内容に関連した試問を実施したところ合格と判断されたので、博士（医学）の学位論文に値するものと認められた。

（総字数 600 字）

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